Ciguatera Food Poisoning
Research Status Report

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ABSTRACT

Ciguatera is a human illness of a sporadic nature associated with the ingestion of a wide variety of coral reef-associated fish that can accumulate through their diet, the heat-stable poison, ciguatoxin. This report focuses on the socioeconomic issues of ciguatera and the status of current endeavors to develop a practical, market-place test to remove ciguatoxin containing fish from commerce. Several difficulties in developing such a test are discussed, including the need to detect trace quantities of ciguatoxin in fish tissues (estimated between 5 and 50 nanograms per test sample or about 0.2 to 2 parts per billion). Immunological methods that possess the desired sensitivity and specificity are under development. Attempts to raise ciguatoxin-specific antibody in immunized animals, however, have been unsuccessful. The lack of sufficient supply of fish toxin is hampering current research, and has lead NMFS and FDA to initiate new in-house programs that focus on culturing suspected toxin-producing dinoflagellates as a source of adequate toxin stock. Although several dinoflagellates are known to elaborate potent toxins, their relationship to ciguatoxin has not been ascertained. If antisera can be raised to these dinoflagellate toxins that will cross-react with ciguatoxin, then the desired immunoasasay to detect ciguatoxic fish should be forthcoming.

INTRODUCTION

An accurate assessment of the incidence of ciguatera poisoning is not available. It has been estimated that 10,000 to 50,000 individuals who inhabit or visit tropical and subtropical areas of the world suffer from ciguatera seafood poisoning each year (Sea Grant Workshop, November, 1983). More recently, however, the illness has not been restricted to

tropical areas. With increasing interstate utilization of valuable tropical reef fish, and rapid air travel by tourists, incidents of ciguatera have been reported in temperate climates (Morbidity and Mortality Weekly Reports, Dec. 19, 1980, CDC, Atlanta, GA).

Ciguatera is one of the most treacherous of the seafood poisons because of the extreme toxicity of the toxin, its sporadic and unpredictable occurrence, and its association with fish considered to be a food staple in many parts of the world. Since most poisonous fish are associated with coral reefs, a wide variety of fish may be involved, presenting a potential impediment to the development of commercial reef fisheries. The marine fish most commonly incriminated are groupers, barracudas, snappers, jacks, king mackerel, and triggerfish. Many other types of reef fish have been reported to harbor the toxin. Lawsuits against seafood wholesalers and restaurateurs alleged to have supplied or served toxin-containing seafoods have significant economic impact. Furthermore, the adverse publicity and out-of-court settlements of the lawsuits have resulted in higher insurance premiums. In Dade County, Florida, where the sale of barracuda has been banned because of past ciguatera episodes, several commercial wholesalers of seafoods are facing the risk of being unable to obtain liability insurance. In addition to barracuda, the sale of amberjack and blackfish is also banned in Puerto Rico; this ban has resulted in a temporary but significant loss of revenue to the island's fishing industry. Thus, ciguatera is a serious economic problem facing tropical and subtropical fisheries, and will continue to plague those involved in the commerce of seafoods until techniques for testing suspect fish are developed and implemented.

Cases of ciguatera were described as early as the 1500's, but our understanding of the disorder still remains fragmentary. The marine microorganism believed to be responsible for the biosynthesis of ciguatoxin, or its progenitor toxin, has been identified as the dinoflagellate, Gambierdiscus toxicus (Yasumoto et al., 1977). Although the precise chemical structure of the toxin is unknown, the suspected toxin has been crystallized recently by Dr. Scheuer of the University of Hawaii and its structure may soon be elucidated. A recent comprehensive review on this subject has been published (Withers, 1982). This report is intended to present those issues that impact directly on the fishing industry and to assess progress in the development of a suitable test to detect ciguatoxic fish at the market place.

Currently, the management of ciguatera outbreaks relies solely on avoiding, if possible, fish species notoriously implicated in the illness and which are commercially harvested from those marine areas suspected of harboring ciguatoxic fish. In the absence of a practical assay for fish toxicity, "contaminated" fish can be estimated only from the reported source of the suspect fish allegedly involved in an outbreak. The absence of an accurate clinical diagnosis makes this approach untenable. Even if a test were available to determine

regional ciguatoxicity or to prophylactically monitor suspect areas, the continuous geographic changes of "poisonous" and "non-poisonous" reefs with time, the migratory patterns of some of the implicated species, and the presumed infrequency of finding the sporadic toxic fish among a large commercial catch would place serious limitations on this approach. With our current knowledge of ciguatera, the most certain and ultimately useful solution would be the implementation of a practical test to determine potential toxicity of individual fish at the market or restaurant level of commerce. The absence of a suitable assay method is without doubt the single greatest impediment to the ongoing national and international efforts to understand the origin, transmission, chemical nature, and pharmacology of ciguatoxin.

RESEARCH APPROACHES

Our present method to determine whether a fish sample contains potentially dangerous quantities of the toxin is the bioassay utilizing mice, cats, or mongooses as the experimental animal. Oral feeding of these animals has presented quantitation problems in the past since unpredictable amounts of toxic flesh are regurgitated. In contrast, intraperitoneal injection of toxic extracts with death of the animal as an endpoint has proved a reliable and reproducible method; it should be noted that mortality (death) is not a typical response of ciguatera in man. The disadvantages of the bioassay are obvious: (1) large numbers of animals are required to quantitate accurately the level of toxin in a single preparation, (2) special animal storage and handling facilities must be constructed and maintained, (3) the assays are time and labor intensive, (4) the commercial availability of the test animals is often unreliable in the geographical regions where testing would most likely be performed, and (5) animal strain differences and environmental factors that may influence the results are difficult to predict or control. Ideally, a laboratory test for ciguatoxin that does not require live animals, but that is rapid, reproducible, sensitive, and quantitative should be designed.

The amounts of ciguatoxin present in fish that pose a public health problem must be approximated so as to select for development an appropriate test system with the required level of sensitivity. Because ciguatoxin is among the most potent, non-protein toxins known to man, researchers have postulated that poisonous fish flesh contain only trace amounts of this offending substance. While ciguatoxin's extreme potency (LD_{50} in mice = 0.45 ug/kg, i.p.; Tachibana, 1980) is of interest to neurophysiologists, its limited amounts in fish tissue adds a new dimension in the development of a test to detect its presence.

One approach in estimating levels of toxin in fish tissue is to correlate a given dose of toxin in mice to the dose a restaurant patron might receive in an average fish serving. Making the liberal assumption that man is equally sensitive to

the toxin as mice, then as little as 36 mg toxin may have a 50:50 chance of producing a human fatality. The possibility has been raised that man may be more sensitive to ciguatera than mice. Based on relative body weight, the dose necessary to produce human symptoms has been suggested (Banner, pers. comm.) to be one-twentieth of a mouse LD_{50} dose. If so, then as little as 1.8 μg may result in the clinical manifestation of ciguatera. In testing suspect fish, only a small portion of the fish tissue can be consumed or otherwise destroyed in the process. A generous test sample could be as large as one ounce (30 g). Thus, one-eighth of a fish serving containing the 1.8 ug ciguatoxin may have 225 ng or less of toxin in the sample to be tested. It must be appreciated, however, that less than 0.1% of ciguatera cases are fatal (Bagnis et al., 1979), and therefore a smaller dose may still give overt symptoms. Using this approach, a more reasonable quantity of ciguatoxin in the 30 g aliquot of a meal that can produce a nonfatal but acute illness may be less than 100 ng. A concentration of 100 ng per 30 g tissue is equivalent to approximately 3 parts of toxin in 1,000,000,000 parts of flesh (3 ppb). Clearly, to detect this amount of ciguatoxin, using this approach, the sensitivity of a market-place test must be very sensitive.

Another approach to estimating the amount of ciguatoxin in ciguatoxic fish is to calculate the amount of pure toxin that has been obtained from fish tisue in purification studies. Tachibana (1980) reported that 50 kg of pooled, moray eel livers provided 1.31 mg of pure ciguatoxin. Assuming no loss during purificaiton, it would follow that the liver tissue contained 78 ng per 30 g of tissue, or about 2.6 ppb.

Since only a very few studies have involved pure ciguatoxin, an approximation of "equivalent purity" must be assigned based on the preparation's biological activity and the determined LD_{50} of pure ciguatoxin at 0.45 ug/kg. McMillan et al. (1980) stated that one gram of flesh from various toxic fish, yielded about 0.75 mg crude extract with an LD_{50} dose response of approximately 2000 mg of crude extract/kg (mouse, i.p.). Hence, one gram of fish flesh yielded 0.00375 of an LD_{50} dose. This equates to 0.17 ng or approximately 5 ng of toxin in a 30 g flesh sample (0.2 ppb).

While these figures are only crude estimates and many unknown parameters influence these calculations, it is obvious that only trace quantities of toxin are present in the flesh of ciguatoxic fish. Therefore, only those detection methods capable of detecting as little as 5 ng of material should be considered.

IMMUNOLOGICAL METHODS

The ability to detect nanogram quantities of ciguatoxin in suspected fish requires a level of sensitivity not possible in most traditional methods of chemical analysis. In recent years, immunological methods have often become the preferred method of analysis, replacing many of the less sensitive and

less specific chemical assays. In diphtheria and other toxin mediated diseases, immunoassays have made possible the definition of "units" of toxins and antitoxins. The recent advances in improved convenience and sensitivity of such immunological assays are impressive. Simple precipitation or agglutination reaction between the test substance and antibody have been replaced by radio-labelled and fluorescent tags. Most recently, enzyme coupled reactions which amplify the detection of antigen-antibody interactions have produced some of the most sensitive and specific assays available. With the increasing use of monoclonal antibodies, a further increase in specificity is being achieved.

It must be assumed, a priori, that any market-place test of ciguatoxic fish will require a minimal amount of tissue extraction of the test sample. As such, any test under development must be capable of identifying ciguatoxin in a very complex chemical mixture, albeit a simple extract or tissue section. Antibodies comprise molecules of biological origin generally posessing a very high degree of structural specificity. This renders them especially suitable for use as "specific reagents" in tests for measuring biologically active substances, e.g. vitamins, hormones, viral particles, protein toxins, etc. (Voller et al., 1981).

In addition to the desired specificity, immunoassays often have sensitivities for detecting as little as 600 molecules (10^{-21} moles; Harris et al., 1979). In general, the traditional precipitation reaction in liquid or semi-solid medium (i.e. single radial immuno-diffusion; Ochterlony) has a sensitivity level of approximately 500 to 1000 ng/ml. In counter-current immunoelectrophoresis, antigen and antibody are driven together under the influence of an electric field, thereby increasing the detection level to about 50 ng/ml (Myhre, 1974). Radioimmunoassay (RIA) is currently one of the most widely applied of all immunological methods. The sensitivities of most RIA methods are less than 0.1 ng/ml (Humphrey and White, 1963), thereby rendering it suitable for ciguatoxin detection. A real advance in convenience and sensitivity was made by the use of enzyme-labelled, instead of radio-labelled, reagents. This permits one to use simple colorimeters rather than gamma-counters which are not always available. In addition, recent innovations in the use of antibody coated beads permits ease of manipulation in simplifying test procedures.

RESEARCH PROGRESS

Bagnis et al. (Progress Report, South Pacific Commission, May 1978), Berger and Berger (1979), Laigret (Progress Report, South Pacific Commission, November 1979), and Hokama et al. (1977) described a radioimmunoassay and an enzyme-linked immunoglobulin assay which used sera obtained from "ciguatoxin immunized" animals. It must be stressed however, that these studies have not as yet provided any conclusive evidence that specific anti-ciguatoxin antibodies are present in the sera obtained from "immunized" animals. In fact, the evidence

presented by Bagnis et al. (ibid.) and Berger and Berger (1979) indicated that no correlation existed between animal toxicity results and positivity in immunoassays developed with Hokama's or their antisera. Studies by Emerson et al. (1983) suggest that the observations of immunological reactions involving countercurrent immunoelectrophoresis may in fact not involve a true anti-ciguatoxin antibody, as reported, but rather a selective binding to serum immunoglobulins. This lack of a specific antibody may be due to several factors, including the chmical nature of the material employed as the immunogen. The currently accepted method for chemical extraction (and partial purification) of ciguatoxin from fish flesh involves partitioning with organic reagents. Because of the toxins's solubility in these reagents, the chemical nature of the extracted material is considered to be lipid, and may therefore prove to be less than an ideal immunogen. From contemporary investigations regarding the immunogenicity of lipids, it is clear that specific antibodies can be produced, although such antibodies may be difficult to detect in the presence of massive non-immunological precipitation of immunoglobulin (Emerson et al., 1983).

The key factor in the development of any immunoassay is the acquisition of high titer, antigen-specific antibodies. The fact that anti-ciguatoxin antibodies have not yet been adequately demonstrated in any laboratory does not preclude the possibility of developing immunoassays. The inability to raise specific antiserum has been hampered due, in part, to the uniqueness of this particular problem.

The first consideration involves the amount of defined material available for immunization of the test animal. Ideally, a minimum of 1 mg of material (assumed to be a "good" antigen) is necessary to successfully immunize and test the resulting antiserum for antigen-specific antibody. Currently, the world's supply of purified ciguatoxin is less than a couple of milligrams. It is obvious that greater emphasis must be placed on acquiring the needed antigen. Recently, several laboratories have focused on establishing a more stable supply of fish toxin.

The second consideration in immunizing an experimental animal is the chemical nature of the antigen. Not all materials make good antigens, and ciguatoxin, an oxygenated polyether compound, would not be expected, a priori, to be a good antigen. In addition, its relatively low molecular weight (est. 1100; Tachibana, 1980) requires that it be coupled with a large molecular weight carrier if the animal's immune system is to recognize the material and develop an antibody response.

The biological nature of toxins possess a rather unique problem for immunologists - animal toxicity. Marine toxins are among the most potent toxins known, active within the ug/kg body weight level. To successfully immunize an animal, the toxins must either be detoxified or a refractory experimental animal must be used. In the case of ciguatoxin with a lethal dose at 0.5 ug/kg as determined by mice injection, a 2.5 kg rabbit is unlikely to tolerate the single milligram of toxin

required to mount an overt antibody response. The lethal dose is about 500-fold less than what might be needed for immunization. It should also be noted that the assumption is made that the rabbit is equally sensitive to ciguatoxin as the mouse. A variety of experimental animals have been tested for sensitivity to ciguatoxin, but a proven ciguatoxin-insensitive, immunologically-component animals has not been discovered. Obviously, some consideration must be given to a chemical or physical treatment of ciguatoxin so as to alter toxicity while maintaining its unique antigenicity.

The suggestion that a particular microorganism might be responsible for synthesizing ciguatoxin found in fish flesh was welcome news for investigators. In addition to providing an explanation on the epidemiological findings of the illness, it opened the possibility of securing reasonable levels of ciguatoxin under laboratory controlled conditions and without the need to extract fish tissues. As a result, progress in ciguatera in recent years has involved our general appreciation of G. toxicus and other dinoflagellates as a potential source of toxic materials. The ubiquitous nature of G. toxicus in tropical and subtropical waters and the capricious nature of ciguatoxic fish has led to the speculation that a simple and direct relationship between the occurrence of this dinoflagellate and ciguatoxic fish may not exist.

The evidence that G. toxicus produces ciguatoxin, the toxin isolated from ciguatoxic fish, is based on several reports in which either wild-cells (Yasumoto et al., 1977; Chungue et al., 1977), or cultured cells (Tindall, 1984) were extracted and fractionated between solvents of different polarities. Unfortunately, these extracts were very crude, and their biological or chemical nature relative to previously reported toxins (e.g., ciguatoxin and maitotoxin) can only be surmised from the non-stringent or non-robust conditions of solvent partitioning and thin layer chromatography employed. The available evidence indicates that cultured G. toxicus does produce at least one toxic component readily distinguished from ciguatoxin on the bases of molecular weight and polarity. This toxin has been tentatively identified as maitotoxin, but its relationship to the toxin first termed "maitotoxin" isolated from surgeon fish (Yasumoto, 1976) is unknown. Nevertheless, the principle toxin elaborated from G. toxicus cultures continues to be referred to as maitotoxin.

The first large scale culture, i.e. 20 liters, of G. toxicus was accomplished at the Hawaiian Institute of Marine Biology by Dr. Withers. The particular single cell isolate capable of growth under their laboratory conditions was originally collected from Tern Island. Since then, several laboratories have succeeded in obtaining and isolating clones of this dinoflagellate. The staff of NMFS has concentrated on about a dozen clones isolated off the Florida Keys while the Southern Illinois University group currently maintains about 20 clones of G. toxicus isolated from the U.S. and British Virgin Islands. Unfortunately, the relative toxicity of only a few of these isolates has been compared. Both the Hawaiian strain and

several of the Floridian strains have comparable mouse toxicity (LD_{50}) of about 15,000 cells/kg. If a single 100 liter culture vat can support the growth of 30 million cells, then it is reasonable to expect enough material lethal for about 1000 mice. To obtain similar toxicity from fish, about 200 pounds of ciguatoxic flesh must be extracted. There is no wonder why so many investigators have turned their attention to these dinoflagellates.

Although the chemical and biological nature of the G. toxicus elaborated toxin is not known, there is little doubt that there exists an association between the dinoflagellate toxin and the toxin extracted from ciguatoxic fish (Sawyer et al., 1984). It is interesting to note a recent report by Dr. Tosteson, University of Puerto Rico (personal communication) that a phototrophic bacterium has been isolated from their unialgal G. toxicus cultures which also exhibits toxicity. Obviously, a great deal of work needs to center on these organisms and their toxins if their relationship to ciguatera is to be understood. Of immediate interest is whether any antisera raised against the dinoflagellate toxins will crossreact with ciguatoxic fish. This is currently being proposed by investigators at the Medical University of South Carolina. Interestingly, Dr. Baden (University of Miami; personal communication) demonstrated some cross-reactivity between his anti-T17 and anti-T34 antiserum (anti-ichthyotoxins from Florida's red tide dinoflagellate) and whole cells of G. toxicus.

OUTLOOK

Although it is recognized that ciguatera is an important seafood illness, it should also be noted that both ciguatoxin and the dinoflatellate toxins are unique biological probes. As neurotoxins, i.e. compounds with a specific action on an animal's nervous system, they are novel and act on either the Na+ or Ca++ channels in rat neuroblastoma cells (Bidard et al., 1984; Takahashi et al.,1983). In recent years, neurotoxins have become very important as molecular probes in the hands of neurophysiologists and neuropharmacologists for studying the molecular mechanisms of nerve action (Narahashi, 1974). These toxins, ciguatoxin and maitotoxin, will surely be in increasing demand for exploring cellular regulatory processes in excitable membranes - including excitation-secretion coupling and excitation-contraction coupling in muscle.

Increasing interest in the chemistry and mode of action of these toxins has put an additional pressure to develop methods for detecting and quantitating the levels of toxin in solution. Obviously, the acquisition of fish toxin is dependent on our ability to identify fish which harbor the toxin. If these methods for detecting ciguatoxin-carriers are amenable to dock- or market-place use, a solution to the ciguatera issues facing the tropical fishermen may be realized. Efforts towards test development with a view to making a useful market-place, screening test must continue. Within the past year, both NMFS and FDA have initiated a collaborative in-house program to

ascertain the chemical nature of these dinoflagellate toxins and to promote the acquisition of antitoxin antibodies. If specific antiserum is obtained, a major hurdle in our efforts to develop a market-place test will have been crossed and the oportunity to resolve the socioeconomic isues of ciguatera that impact on the commercial fishing industry will be much closer to reality.

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